PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

KIMBERLY F. GLASSMAN ET AL CASE NO.: BB1449 US NA

APPLN. NO.: 09/887194 GROUP ART UNIT: 1635

FILED: June 22, 2001 EXAMINER: ASHEN, JON BENJAMIN

FOR: RECOMBINANT CONSTRUCTS AND THEIR USE IN REDUCING GENE EXPRESSION

Date: October 11, 2005

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Dr. Johan Stoop Pursuant to 37 CFR §1.132

I, Johan Stoop, am a citizen of Belgium, residing at 8 Farm Avenue, Wilmington, De, United States of America, and I declare as follows:

1. I am a graduate of North Carolina State University (Ph.D. in Plant Physiology 1993, M.S. in Horticulture, 1989) and a graduate of the Katholieke Universiteit Leuven, Belgium (M.S. Agriculture Engineer, 1987). I have been employed by E. I. du Pont de Nemours and Company since 1998 directing and conducting research in plant genetic engineering. I am working under the supervision of Dr. Anthony Kinney, one of the co-inventors of the above-identified application.

2. I have reviewed the Office Action dated April 21, 2005 and am aware that this declaration is being submitted to address the concerns set forth on

pages 5 through 12 of the aforementioned Office Action. Specifically, this declaration is being used to address the rejection of claims 46-52 (now claims 53-59) under 35 USC §112, first paragraph, as failing to comply with the written description and enablement requirements.

3. Example 10 which is set forth on pages 40 through 43 of the instant specification, describes the suppression of soybean galactinol synthase genes using constructs comprising ELVISLIVES. Specifically, a plasmid construct was assembled containing fragments of two galactinol synthase (Gas) soybean genes, 390 bp of GAS1 and 399 bp of GAS 2. The two Gas fragments were cloned into the NotI site of a 2XEL cassette. The promoter region was a late embryo (Lea) promoter from soybean. The entire cassette containing the Lea promoter, 2XEL, the two GAS fragments and the phaseolin 3' end were cloned into the BamHI site of vector pKS136 to create vector pKS149. The complete EL region of pKS149 is shown in SEQ ID NO: 29. Since pKS136 also contained a part of the FAD2 gene under the control of the Kti promoter, a concomitant reduction of FAD2 and GAS expression was expected in transformed soybean seeds compared to the levels of expression of FAD2 and GAS in untransformed soybean seeds. Suppression of FAD2 and GAS was followed by measuring the levels of the substrate (oleic acid in the case of FAD 2 suppression) and products (raffinose family oligosaccharides in the case of GAS suppression). It was found that suppression of Fad 2 and GAS led to a high oleic and low raffinose oligosaccharides phenotype in the transformed seeds compared to untransformed seeds. Thus, the desired outcome was achieved.

4. Alternatively, another way to monitor suppression of these endogenous genes, FAD 2 and GAS, is by measuring the level of specific RNA expression. This can be done using quantitative Real Time PCR. Specifically, quantitative Real Time PCR of Soybean seeds transformed with KS149 (see above) was performed and the results were compared to a control line (Jack) whose seeds were not transformed with KS149. RNA expression of GAS1, GAS2 and a third galactinol synthase, GAS3, was quantified using three different methods. An absolute quantification method, wherein the absolute amount of mRNA of GAS1, GAS2 and GAS3 in untransformed seed (Jack) and transformed seed is measured against a standard curve, made from plasmid DNA of each of the respective sequences. Fold change and % reduction of mRNA expression in

the transformed versus the control seeds was calculated based on the absolute values. Relative quantitation was performed using a comparative cycle threshold method and a relative standard curve method. These methods determine the relative amount of mRNA in the transformed seeds compared to the untransformed ones, normalized against three different endogenous genes. All of the methods showed similar results, wherein the expression of GAS1, GAS2 and GAS3 were reduced by 73%, 84% and 39%, respectively, in transformed seeds compared to control (untransformed) seeds. The results obtained by the three methods are summarized in Table 1 attached hereto.

5. Appendix A, attached hereto, sets forth an alignment of the GAS1 and GAS2 fragments from vector KS149 with the full length coding sequence of GAS3. The percent identity between the GAS1 fragment and GAS3 is 66.9% and the percent identity between the GAS 2 fragment and GAS3 is 70.5%.

6. One skilled in the art would inexorably conclude that since the sequence identity between the GAS1 and 2 fragments and the full length sequence of GAS3 is about 70% and the observed suppression of GAS3 was 39% as discussed in paragraph 4 above, then it would be reasonable to expect that RNA sequences homologous to all or part of the target mRNA would function to reduce the expression of any target mRNA or any endogenous RNA expressed in soybean that has **at least** 80% sequence identity with RNA sequences homologous to all or part of the target mRNA.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Johan Stoop

October 17 2005
Date

Appendix A

Appendix A shows an alignment of the nucleotide sequences of fragments of GAS1 (390 bp from nucleotides 13-402 of SEQ ID NO:30), Gas2 (390 bp from nucleotides 129-527 of SEQ ID NO:32) and the full length GAS3 gene coding sequence. Nucleotides conserved among all sequences are indicated with an asterisk (*) on the top row; dashes are used by the program to maximize alignment of the sequences.

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      *       *       * *       *                       *
GAS3  A T G G C T C C T G A A C T T G T C C C C A C C G
GAS1  A T C A C C A C T G - - - T C A A A A C C A C C A
GAS2  A C C A C C G T T G - - - T T G C C A A T G T C A

                          * *                       * *
GAS3  T T G T G A A A T C C A G T G C T G C G T T C A C
GAS1  T C A - - - - - - C C G A C G C T C A A G C C A -
GAS2  C C A - - - - - - C C G A G C A A T T A C C C A -

        *                                       *   * * *
GAS3  G A A A C C C G C G A C C C T T C C A A G G C G T
GAS1  - A G G T C G C C A C C G A T C A T G - G T C G T
GAS2  - A G G C T C G T G G A G G A A G T G - G G C G T

      * * * *   * * *   * *   * *   * *   * *   * *   *
GAS3  G C C T A C G T G A C A T T C C T C G C C G G A A
GAS1  G C C T A C G T C A C C T T C C T C G C C G G A A
GAS2  G C C T T C G T G A C C T T T C T T G C T G G G A

      * * * * * * *   * *   * *   * *   * *   * *   * *
GAS3  A C G G T G A C T A C G T G A A A G G G G T G G T
GAS1  A C G G T G A C T A T G T G A A A G G T G T C G T
GAS2  A C G G T G A T T A C G T A A A G G G T G T C G T

        * *     *   * *   * * * * *     * *   * * * *
GAS3  T G G C C T C G C C A A A G G G T T G C G A A A G
GAS1  T G G C T T G G C A A A A G G T C T G A G A A A A
GAS2  G G G T T T G G C C A A A G G A C T G A G A A A G

      *     * *   *   *     * * * * * *     * * * * * *
GAS3  G T G A A A A C C G C G T A C C C G T T G G T G G
GAS1  G T G A A G A G C A T G T A C C C T C T G G T G G
GAS2  G C C A A A A G C A T G T A C C C T T T G G T G G

      *   * *   * *     *   * *   * * * * *   * *     *
GAS3  T G G C T G T C C T C C C C G A T G T G C C G G A
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GAS1  T T G C A G T G C T A C C C G A T G T T C C C C A
GAS2  T T G C T G T G T T A C C A G A T G T T C C T G A

        * *   * *   * *     *   * *   * *         * *
GAS3  G G A G C A C C G T A A G A T C C T G G A G T C T
GAS1  A G A T C A C C G C A A C A T T C T C A C C T C C
GAS2  A G A A C A T C G T G A G A T T C T C A A A T C C

      * *   * *   * * * * *   * *         * * * * *   *
GAS3  C A G G G C T G C A T C G T T C G C G A G A T C G
GAS1  C A A G G T T G C A T T G T T A G A G A G A T T G
GAS2  C A A G G T T G C A T T G T C A G G G A G A T T G

      *   * *   * *   * * * * *   * *   * *   * *   * *
GAS3  A A C C C G T T T A C C C A C C C G A A A A C C A
GAS1  A G C C C G T G T A C C C C C C A G A G A A T C A
GAS2  A A C C T G T G T A C C C T C C T G A G A A C C A

        * * * * * * * * *   *   * * * * * *   * * * * *
GAS3  A A C C C A G T T T G C C A T G G C T T A T T A C
GAS1  A A C C C A G T T T G C C A T G G C A T A T T A C
GAS2  G A C C C A G T T C G T C A T G G C C T A T T A T

      * * * * * * * * *   * *   * * * * * * * * *   * * * *
GAS3  G T C A T C A A C T A C T C C A A G C T C C G T A
GAS1  G T C A T C A A C T A T T C C A A G C T A C G T A
GAS2  G T C A T C A A T T A C T C C A A G C T A C G T A

      *   * * * * * * * * *   * * * * * * * * * * *     * *
GAS3  T A T G G G A G T T T G T G G A G T A C A G C A A
GAS1  T T T G G G A G T T T G T G G A G T A C A G C A A
GAS2  T T T G G G A G T T C G T G G A G T A C A A G A A

      * *   * * * * * * * *   *   * * * * *   * *   * *
GAS3  G A T G A T A T A C T T G G A C G G A G A C A T T
GAS1  G A T G A T A T A C C T A G A C G G T G A T A T C
GAS2  G A C G A T A T A C C T A G A C G G T G A C A T C

        *   * *   *   * *     * * * * *   * * * * * *
GAS3  G A G G T A T A T G A G A A C A T A G A C C A C C
GAS1  C A A G T T T T T G A C A A C A T T G A C C A C T
GAS2  C A A G T A T T T G G A A A C A T A G A C C A C T

      *
GAS3  T A T T T G A C C T A C C T G A T G G T A A C T T
GAS1  T G
GAS2  T G T T T G A T C T G
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GAS3 T T A C G C T G T G A T G G A T T G T T T C T G C

GAS3 G A G A A G A C A T G G A G T C A C A C C C C T C

GAS3 A G T A C A A G G T G G G T T A C T G C C A G C A

GAS3 A T G C C C G G A G A A G G T G C G G T G G C C C

GAS3 A C C G A A T T G G G T C A G C C C C C T T C T C

GAS3 T T T A C T T C A A C G C T G G C A T G T T C G T

GAS3 G T T C G A A C C C A A C A T C G C C A C C T A T

GAS3 C A T G A C C T A T T G A A A A C G G T G C A A G

GAS3 T C A C C A C T C C C A C C T C G T T C G C T G A

GAS3 A C A A G A T T T C T T G A A C A T G T A C T T C

GAS3 A A G G A C A T T T A C A A G C C A A T C C C T T

GAS3 T A A A T T A C A A T C T T G T C C T C G C C A T

GAS3 G C T G T G G C G C C A C C C G G A A A A C G T T

GAS3 A A A T T A G A C C A A G T C A A G G T T G T T C

GAS3 A C T A T T G C G C A G C G G G G T C C A A G C C

GAS3 A T G G A G A T A T A C G G G G A A G G A A G A G

GAS3 A A T A T G C A G A G G G A G G A C A T A A A G A

GAS3 T G C T G G T G A A G A A A T G G T G G G A T A T

GAS3 C T A C A A T G A T G C T T C G C T T G A C T A C

GAS3 A A G C C A T T G A T G A A T G C A A G T G A A G

GAS3 C T C C A G C A G C G G A T G G T G T T G A C A T

GAS3 T G A A C A A T T C G T G C A G G C T C T A T C A

GAS3 G A G G T T G G T C A T G T T C A A T A T G T C A

GAS3 C C G C G C C T T C A G C A G C T

Table 1

	Fold Change[1]		%Reduction[2]	
	Mean	Stdev[3]	Mean	Stdev[3]
GAS1	0.27	0.1	73	6
GAS2	0.16	0.1	84	5
GAS3	0.61	5.7	39	16

[1]Fold Change of mRNA from seeds transformed with KS149 compared to control (Jack)
[2]% Reduction in mRNA from seeds transformed with KS149 compared to control (Jack)
[3]Stdev=Standard deviation